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THE INDUCTION OF EGG-LAYING IN THE SALAMANDER, *EURYCEA BISLINEATA*, BY PITUITARY TRANSPLANTS

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It has been established by Smith (1926, 1927), Smith and Engle (1927), Zondek and Aschheim (1927) that frequent transplants of the pars anterior of the pituitary induce a precocious sexual maturity in rats and mice. However, Riddle and Flemion (1928) found that similar treatment with daily homeotransplants of anterior pituitary in doves had only a slight effect on the female reproductive cycle. Wolf (1929) showed that the common frog, *Rana pipiens*, could be induced to lay its eggs in November by transplanting fresh anterior lobe substance into its lymph sacs on several successive days. Daily transplants also induced sexual activity in the male. Wolf (1929a) was able to induce egg-laying and fertilization in this frog in the latter part of October, several months before the normal breeding season.

The present paper represents a preliminary report of a study of the effect of anterior pituitary transplants on ovulation in salamanders. In brief, we find that anterior pituitary transplants have the same effect in salamanders as Wolf reported in frogs. The species which we selected for this work is the common two-lined salamander, *Eurycea bislineata* (Green), a species which breeds in May and June (Dunn, 1926). The experiments were carried on during November, December and January, with material freshly collected at Bayside, L. I. Several hundred specimens of *Eurycea bislineata* have been kept for varying periods during the three months in our laboratory tanks but none have ever laid their eggs spontaneously.

In operating, the pars anterior of the pituitary was removed from a freshly killed adult *Eurycea bislineata*, either male or female, and placed in .7 per cent salt solution. An adult female was chloretonized in a 1 to 2000 solution and a small slit made in the skin covering the ventral surface of the tongue musculature. The isolated pars anterior was thrust posteriorly through the slit to the vicinity of a blood vessel. The anæsthetized animal was next placed in water, a stitching of the wound being found unnecessary. The controls were treated in a similar manner, but a small piece of muscle, the same size as the pars anterior, was inserted in the lymph space underlying the tongue musculature.

Five specimens each received a single transplantation of anterior pituitary on December 28. Within two days one of this series laid 24, another 25, another 36, and another 3 eggs. None of the 4 controls laid eggs. Four other adult females each received four transplants of anterior pituitary over a period of four weeks, approximately one transplantation a week, beginning November 22. Of this group, one individual deposited 28, another 23, and another 9 eggs. There were 3 controls in this series. Six specimens each received one single transplant on January 2, and one double (2 entire pars anterior) transplant on January 4. Of these, one female laid 25, one 23, and one 3 eggs. Seven individuals received one double transplant each on January 4, and within 48 hours one animal laid 17 eggs and two more 36 and 15, respectively. Four individuals each received six transplantations of anterior pituitary, one a week beginning November 20, but none of this group laid eggs. The failure of the last series to respond may be accounted for by assuming that the movements of the salamander displaced the graft or that the transplant was too rapidly absorbed in these cases. Wolf (1929) found considerable irregularity in the response of *Rana pipiens* to anterior pituitary transplants. The fact, however, that a large percentage of females do lay eggs after one or more transplantations, while none of the controls or untreated animals respond, is definite evidence of the effect of anterior pituitary substance on the ovulation of both frog and salamander.

Wolf (1929a) found that transplanting the anterior pituitary into adult male *Rana pipiens* in the fall induced the sexual activity of that sex. It has been shown by Noble and Weber (1929) that courtship in *Eurycea bislineata* normally takes place in the fall and hence experiments with the male were not attempted. The female *E. bislineata* picks up the spermatophores in the fall and the spermatheca during this period is frequently full of spermatozoa. Hence, eggs laid in the fall induced by pituitary transplants would presumably develop normally, although our experiments have not been carried far enough to establish this point.

The females of *E. bislineata* receiving the transplants were kept in large crystalizing dishes, each provided with a flat stone raised on smaller stones a centimeter or less from the bottom of the dish. Each dish was filled with water to a depth of two centimeters. In nature, the species breeds in brooks, attaching its eggs to the under side of stones, usually in running water. The artificial character of the breeding dishes did not prevent the egg-laying from proceeding normally. The eggs were attached singly to the underside of the flat stone, the female moving to a new position after each egg was deposited. In a few cases the

animal crawled to the top of the rock for two or three minutes between the laying of each egg. The entire process occupied about one hour. Generally the eggs were attached near the edge of the rock, the tail of the female curling out and up over the edge. The actual laying of each egg required from one to two minutes and was accomplished by the animal pressing the cloaca against the surface of the rock.

The fact that normal egg-laying of salamanders may be induced in the laboratory by anterior pituitary treatment should be of considerable interest to students of amphibian life histories. There are a number of species whose egg-laying has never been described. Adults of these species may be collected frequently. If these species are subjected to anterior lobe treatment it is presumed from our experiments on *Eurycea bislineata* that egg-laying may be induced and will proceed normally if facilities are provided.

CONCLUSIONS

The transplantation of one or more anterior pituitaries in *Eurycea bislineata* induces egg-laying several months before the normal breeding season.

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